EVALUATION OF DIFFERENT LABORATORY TECHNIQUES FOR DETERMINING SEED VIGOR IN FORAGE AND GRAIN SORGHUMS

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INTRODUCTION

Germination of seeds in accordance with the rules set forth by the American Association of Offical Seed Analysts and the International Rules of Seed Testing, does not satisfactorily indicate the performance of the seed under field conditions. The standard laboratory germination test measures the ability of seeds to germinate under artificial, but ideal conditions. Seeds planted under field conditions are subjected to many variables that may adversely affect stand establishment.

Within the past few years considerable work has been directed toward methods of determining the planting quality of seeds. Recently many types of direct and indirect vigor tests have been developed. The cold test for evaluating planting quality in seed corn is an outstanding example. Cold tests attempt to simulate, under laboratory conditions, the adverse conditions seed frequently encounter under field plantings.

The objectives of this study were: (1) to determine the effectiveness of artificially applied adverse treatments in evaluating the seeds of several varieties and hybrids of both forage and grain sorghums; and (2) to measure the effect of different moisture-stresses on germination and vigor. The latter objective was omitted because of the unexpected size and complexity of the first objective.

REVIEW OF LITERATURE

This review attempts to organize the more important research finding pertaining to vigor tests of seed in Europe and the United States. Problems directly and indirectly related to vigor are also surveyed.

Many factors affect seed vigor and performance, and it is unlikely a test can be devised that will meet the requirements for all seeds and conditions. The following causes for the lack of vigor were gives by Schoorel (32): (1) unfavorable weather conditions occurring prior to and at the time of seed harvest; (2) careless handling of the seeds after and during harvest; (3) prolonged storage under unfavorable conditions; (4) the presence of and activity of parasitic organisms; (5) chemical treatments during storage; and (6) the genetic properties of the seed. Quimby et al. (28) reported the importance of the source of seed on germination, yield, winter hardiness, and disease resistance of hard red winter wheat. Three additional factors very likely affecting germination were pointed out by Fox and Albretch (12): (1) soil fertility and its influence upon the elemental composition of the seed produced; (2) the chemical and biochemical properties influencing the cellular metabolism of the germinating embryo; and (3) the metabolic activity of the seed and the nutrients available to the germinating embryo. Both laboratory and field tests conducted by Pinnell (27) indicated a relationship between vigor and the genetic composition in corn. He observed that the germination vigor of double crosses (hybrids) was better than single cross hybrids and that the single cross hybrids were more vigorous than inbred lines.

Vigor tests have been catalogued into two groups by Isely (18): (1) direct tests which simulate pertinent unfavorable field conditions on a laboratory scale; and (2) indirect tests which measure certain physiological attributes of seeds. Most of the emphasis on vigor tests in the United States has been on tests of the direct type. Isely (17) developed the popular cold test for evaluating hybrid seed corn in the United States. The principle employed in this kind of test may be applicable to a number of crops. According to Tempe (38) a cold test is mainly adapted to sub-tropical crops and suggests the possibility of using a high temperature test for certain other crops. A method recommended by Rice (30) involved planting the seed in a mixture of sand and Pythium-infested soil and exposing the test replicates to a 10°C. temperature during part of the germination period followed by a transfer to a near optimum germination temperature. In addition, he suggested that a cold test should be of value in evaluating the effectiveness of seed fungicides and seed processing methods. Imbibing the seeds on a moist substrate in a warm environment for 24 hours prior to a cold treatment of 2 to 3 days at 10°C., enabled Reddy and Gerhold (29) to obtain results equivalent to a 7 to 8 day cold test without a pre-imbibition period. They concluded that treatment with fungicide does not entirely prevent attack by Pythium if moisture conditions are favorable. In addition they observed that a 50 to 60 percent capillary soil saturation gave the greatest suppression of germination.

Investigators Tatum and Zuber (37), Ho (13), Reddy and Gerhold (29), Hoppe (15), and Hooker and Dickson (14) have indicated that Pythium spp. are primarily responsible for rotting of corn seed under low temperature conditions.

Rather extensive reviews were made by Crossier (10), Isely (18), (19) and Svien and Isely (34) of literature concerning cold tests and its relative merit as a vigor test. They pointed out that the cold test has been difficult to standarize because of differences in temperatures, soil conditions and especially soil micro-organisms. These people all agree that a cold test is not a test for cold resistance but rather a test for resistance to seed rotting fungi which attack slowly germinating seeds in cold soils.

Results of separate studies conducted by Clark (8) and Hoppe (15), have indicated that the cold test can be used to advantage in predicting relative field performance of early spring plantings of sweet corn.

A number of other vigor tests have been suggested. The most interesting is a water-soaking test described by Tatum (36), in which corn seed from different lots were soaked in water. He found a correlation between the turbidity of the steep water between lots and the results obtained by the cold test for corn. The very permeable seeds showed a high concentration of solid material leached out during the soaking period. These seeds were found to be susceptible to cold conditions.

Vigor is defined by Isely (19) as the sum total of all seed attributes which favor stand establishment under unfavorable conditions. A more practical concept of vigor was proposed by Delouche and Caldwell (11). They stated that vigor is the sum of all seed attributes which favor rapid and uniform establishment under field conditions.

Vigor tests used in Europe were reviewed in papers by Delouche and Caldwell (11), Isely (18), Schoorel (32) and Tempe (38). The indirect tests were found to be essentially the main types used. These were classified (11) into four general groups as follows: (1) Biochemical tests - The use of tetrazolium as a means of evaluating vigor; (2) Growth rate tests - Speed of germination and rate of seedling growth (measured as dry weight produced); (3) Stress-tests - Reaction of seeds to stress conditions such as unfavorable temperatures and moisture levels exposure under vacuum, soaking in NaOH and hot-water, and mechanical barriers; and (4) Dark-test - Growing the seedlings in darkness until exhaustion, followed by measuring sprout length.

Apparently it is not the weight of the covering layer over the seed zone (38) that affects germination and emergence, but instead, it is the layer thickness.

A formula for evaluating speed of germination as a function of vigor was developed by Throneberry and Smith (39). The formula consists of taking the number of normal seedlings per 100 units counted each day, times the reciprocal in days required. Maquire (24) suggested a method that would, in his opinion, benefit forage breeding programs using the concept of speed of germination in vigor tests.

A relationship was found by Nutile and Hackett (26) between vigor, as measured at the first count in blotter tests, and emergence of normal seedlings in soil tests under laboratory and greenhouse conditions. However, in a study of substrate moisture conditions in folded blotter paper and Petri dishes Isely (20) reported the substrate were found to be highly variable even in humidified germinators.

As early as 1926 Swanson (35) working with sorghums reported differences in seed coat permeability within feterita, blackhull kafir, red amber and Kansas orange. He attributed this difference between the varieties to the differences in number and structure of cells in the seeds. Similarly, Stiles (33) showed that varietal differences exist in water absorbing capacity of seeds of cotton, corn and beans. Burch and Delouche (7) found that different kinds of seeds as cotton, soybean, castor bean and oat varied in their requirements of moisture content for germination. They found in addition, that the Cotyledon absorbed more moisture in relation to their dry weight than did endospermous tissue.

A considerable volume of work can be found recommending the use of tetrazolium as a test for germination and vigor. A significant correlation was found by Metzer (25) between the results of the tetrazolium tests and actual germination and emergence of cotton seed. Copeland et. al. (9) felt that the use of 2, 3, 5-triphenyl-tetrazolium chloride as an indicator of the germination capacity of seed would reveal much more about the physiological condition of the seeds than would normally be detected by a standard germination test. However, the validity of the tetrazolium test is dependent upon the ability of the individual to interpret the stain results. It is not a magic miracle test that can be conducted by anyone.

As reported by Iljin (16), certain physiological processes are influenced by drought. He stated that the processes most affected were the functions of the stomata, photosynthetic and respiration processes, the metabolism of carbohydrates, and osmosis.

A standard laboratory test (9) evaluates the germination capacity of the seeds at the time the test is conducted. However, as normally interpreted it does not reveal physiological weaknesses within the cell structures that may be deterimental to the continuing health of the seeds.

Toole, et al. (40) suggested that favorable effects of warm constant temperatures may result from the creation of a balance of the intermediate materials of respiration at the high temperature part of the cycle, which may be unfavorable for germination, but may promote germination at the lower one. Species and varieties according to Koller, et al. (21) differ in their capacity to germinate at light water tensions and this capacity may depend on the germination temperature. He reported further that similar differences may exist in the capacity of seeds to germinate at very low water tensions. Favorable effect of repeated cycles of imbibition and drying on the subsequent rate of germination and seedling vigor are correlated with increased water absorbing capacity, probably caused by an increased amylase activity.

The size of sorghum seeds was found, by Robbin and Porter (31), to have an influence on seedling vigor. Brown, et al. (6) presented data on the germination of more than 500 sorghum samples to different temperatures under various storage conditions at different intervals of time after harvest. They concluded that dormancy is much less common in sorghum than in barley or oats.

In studies on effect of low temperature on the germination of artifically dried seed corn, Livingston (22) showed that seedling emergence has an inverse relation to kernel moisture content at the time of harvest. In his studies, artificial drying of the seed intensified this effect, particularilarily in non-sterile soil. Data reported by McRostie (24) indicate that when corn ears were dried on the stalk to around 30 percent moisture, no appreciable damage was caused by the use of drying temperatures of 130^{o}F. Above a 50 percent seed moisture level damage was evident at all drying temperatuers above 105^{o}F.

In storage studies carried out by Toole and Toole (42), they observed that at the start of experiments, when germination of the seeds is high, there is little variation. The variation depends on the sampling of live and dead seeds. However, as the seeds approach the 50 percent level of viability, variations among replicates are larger. It is recognized that the sampling variation at the 50 per cent level is greater than at the 90 percent level.

According to the rules for testing seed (2, 3, 4, 5) in a seed laboratory, germination is defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seeds in question, are indicative of the ability to produce a normal plant under favorable conditions. Normal seedlings are those which possess those essential structures that are indicative of their ability to produce plants under favorable conditions. Abnormal seedlings are all seedlings that do not permit classification as a normal seedling. Seedlings covered with fungi or bacteria are regarded as normal if they are otherwise normal. A seedling that has been seriously damaged by bacteria or fungi from any source other than the specific seed should be regarded as normal if it is determined that all essential structures are present. If a chemical preparation is used to reduce the spread of micro-organisms, the results are to be regarded as supplemental information and reported as such.

METHODS AND MATERIALS

Twenty-four different sorghum accessions were obtained from the sorghum section of the Oklahoma State University Agronomy Department for studies to be conducted in the U.S.D.A. Grass Seed Research Laboratory on the campus. The accessions, as shown in Table 1, represent both forage and grain types that are presently produced, or are being evaluated experimentally in Oklahoma.

TABLE I

ACCESSIONS OF SORGHUM STUDIED AND THE AREA OF ADAPTATION IN OKLAHOMA.

Varieties	Type		Oklahoma	
	Forage	Grain	East	West
1. Redlan	-	X	X	-
2. White Wheatland	-	X	-	X
3. African Millet	X	-	-	X
4. Sumac 1712	X	-	X	-
5. Redbine 66	-	X	-	X
6. Bonar Durra	X	X	X	X
7. Kansas Orange	X	-	X	-
8. Sugar Drip	X	-	-	X
9. Redbine 60	-	X	-	X
10. Texas Blackhull	-	X	-	X
11. Sharon Kafir	-	X	-	X
12. Cache Feterita	-	X	-	X
13. RS-681/1	-	X	-	X
14. NB-505	-	X	-	X
15. RS-608	-	X	-	X
16. AKS-614	-	X	-	X
17. RS-661	-	X	-	X
18. OK-613	-	X	-	X
19. OK-632	-	X	-	X
20. NB-504	-	X	-	X
21. OK-612	-	X	-	X
22. RS-650	-	X	-	X
23. RS-610	-	X	-	X
24. RS-630	-	X	-	X

/1 Refers to accession number of hybrids

Recognizing the probability that differences in seed size, growth habit and age of the seeds being studied were variables that could possibly lead to erroneous conclusions, the decision was made to characterize each variety by some measure of vigor.

The vigor test for seed corn (11) is the only test extensively used in the U.S.A. to evaluate planting quality. It has been shown in numerous studies (8, 9, 38(that a standard laboratory germination test does not reflect true planting quality in all cases. Seeds when planted under field conditions are subjected to many variables that affect stand establishment. Only well mature, fresh seeds with the reserve energy required to tolerate these variables are able to survive and produce seedlings.

Since there is not a recommended and/or standard method for evaluation of seedling vigor or seed quality for sorghums, four treatments were selected for study. Three of them differed only in the substrate material and one was entirely different from the others. The treatments used in this study were:

1. Uniformly placing the seeds on six thicknesses of kimpak tissues moistened with 20 ml. of water and subjecting them to 5-10°C. temperature for five days before transfer to a warm environment.
2. In addition to the above treatment, a one inch layer of vermiculite moistened with 150 ml. of water, was placed over the seed in this treatment and then the seed was subjected to 5-10°C. temperature for five days before transfer to a warm environment.
3. This treatment was the same as number two except for the use of a one inch layer of sterile sand moistened with 60 ml. of water, instead of vermiculite.

4. The fourth treatment utilized a hot-water (100°C.) treatment of five seconds prior to placing the seeds on moistened kimpak tissue. This treatment did not receive a cold incubation period and was placed directly, following treatment, into the constant 30°C. germination chamber.

The preparation of the treatments was conducted in such a manner that all treatments within one replication were placed in the germination environment on the same day.

These tests have been used in evaluating seed quality previously by other workers (17, 30, 39) with good results.

The recommended number of seed-units per replicate in standard germination tests is 100. Because of the large number of seeds required and the problem of incubation space, it was necessary to reduce the number of seed units to 25 seeds per replication of each treatment.

Unpublished data on seed population, or sample size required for studying the germination requirements of oats (Avena sativa), indicate that 50 and even 25 seed units per replicate under controlled conditions gave satisfactory results. In these tests the average germination was found to be within 1 to 5 percent of that obtained when germination was conducted with the accepted number of 100 seeds.

Notes were taken at daily intervals on each treatment and variety by replication. Data on the speed of germination were taken at the time germination counts were made. Vigorous seedlings were determined at the end of each germination test according to the relative rate of growth among the different varieties. In every case a seedling was considered normal when, in addition to having normal structures, the plumule had split the coleoptile. The distinction between a normal and an abnormal seedling was made following the concepts of other workers (2, 3, 4, 5) for treatments 1 and 4. The rate of germination was calculated by dividing the number of seedlings obtained at each count by the number of days required as suggested by Thorneberry and Smith (39).

Because of the large number of germination boxes required (24 varieties X 3 replications X 4 treatments = 288) and limited space within a standard germinator, a statistically unusual incomplete block design was used. One replicate, representing a complete set of the four treatments applied to each of the 24 varieties, was placed in the germination environment at five day intervals for a period of 15 days. A total of 12 shelves were used in a 30°C. environment, with each shelf containing 8 boxes representing four varieties and two treatments. The uniformity trials on germination of switchgrass seed conducted by Ahring et al. (1) were used as a basis for the design employed in this study. Varieties were divided into six groups with four varieties in each group. Through the use of this design comparisons made between varieties within a group were not confounded with shelves. Statistical confounding in each replication was different as shown in Table 2, as this type of design gave unconfounded contrasts. The comparisons were made for speed of germination and vigor within and between varieties and groups by replication.

TABLE II

CONFOUNDING VARIETAL GROUP PER SHELF BY COMPARISON OF ALL POSSIBLE COMBINATIONS OF TREATMENT VS. TREATMENT BY REPLICATION

	Treatment Vs. Treatment					
Varieties	1 vs. 2	1 vs. 3	1 vs. 4	2 vs. 3	2 vs. 4	3 vs. 4
1-2-3-4	Rep-2	Rep-3	Rep-1	Rep-1	Rep-3	Rep-2
5-6-7-8	" "	" "	" "	" "	" "	" "
9-10-11-12	" "	" "	" "	" "	" "	" "
13-14-15-16	" "	" "	" "	" "	" "	" "
17-18-19-20	" "	" "	" "	" "	" "	" "
21-22-23-24	" "	" "	" "	" "	" "	" "

The interaction of the varieties within a given contrast are not confounded, as shown in Tables 7, 8, and 9 of the appendix.

RESULTS AND DISCUSSION

Throughout the study significant differences existed among treatments, replications, and shelves, as indicated in Table 3. Of the six-groups, significant differences among varieties were found within three groups, and in two of thes (groups 3 and 4), signigicant interactions were measured between varieties and treatments. Differences existed among varieties within groups and among treatments, in both the speed of germination and the objective vigor measurements.

Normal Germination

A normal seedling is defined (3) as those seedlings possessing the essential structures that are indicative of their ability to produce plants under favorable conditions. In sorghums, seedlings having well-developed roots and plumules are considered normal. It is not necessary for the plumule or leaf-roll to have ruptured the coleoptile for a seedling to be considered normal, but it must have a plumule that is visibly over one-half the length of the coleoptile. This criterion explains perhaps the large differences found in normal germination between treatments as shown in Figure 1. Seedlings were readily observed where kimpak tissue was used (treatments 1 and 4), but were hidden from view under 1-inch layers of sand and vermiculite (treatments 3 and 2, respectively). This in itself makes the observations on such treatments relative, but does give an indication of the energy of germination with and without a layer of material over the seed-zone following an adverse treatment.

The measurement of vigor was found to be a highly relative factor with results being closely associated with the type of substrate used and the amount of fungi infestation occurring during the pre-chill and incubation periods. In every case the order of rank by treatment per group in percent normal germination was 1, 4, 3, 2. A 1-inch layer of sand and/or vermiculite over the seed zone, plus subjecting the treatments to 10°C. for five days prior to transfer to an optimum environment, was sufficient to reduce the normal germination count (Figure 1) by as much as 75.3 percent.

TABLE III

ANALYSIS OF VARIANCE OF PERCENT NORMAL SEED GERMINATION OF SORGHUM VARIETIES AND HYBRIDS BY GROUPS

Source	Group 1 (Var. 1, 2 3, 4)			Group 2 (Var. 5,6,7,8)		
Replicates	2	3,508.0	14.616**	2	1,216.7	5.5329*
Shelves in Reps.	3	1,985.0	8.270	3	1,683.4	7.6552**
Varieties	3	356.7	1.485 N.S.	3	258.7	1.1764 N.S.
Treatments in Shelves	6	1 857.0	7.731**	6	2,843.7	12.9317**
Treatments X Var. in Shelves	18	310.0	1.292 N.S.	18	171.3	.7789 N.S.
Varieties X Shelves	15	240.2		15	219.9	
	Group 3 (Var. 9,10,11,12)			Group 4 (Var. 13,14,15,16)		
Replicates	2	856.3	3.310 N.S.	2	901.3	8.3299**
Shelves in Reps	3	1,215.0	4.696*	3	1,226.6	11.3364**
Varieties	3	1 728.3	6.672*	3	596.0	5.5083*
Treatments in Shelves	6	1 170.3	4.5237*	6	457.0	13.4658**
Treatments X Var. in Shelves	18	1 925	7.4429**	18	360.1	3.3280*
Varieties X Shelves	15			15		
	Group 5 (Var. 17,18,19,20)			Group 6 (Var. 21,22,23,24)		
Replicates	2	2,468.1	14.1600**	2	2,366.7	28.2422**
Shelves in Reps.	3	784.1	4.4985*	3	2,466.3	29.4307**
Varieties	3	1,047.0	6.0068*	3	161.0	1.9212 N.S.
Treatments in Shelves	6	2,020.9	11.5943**	6	962.3	11.4832**
Treatments X Var. in Shelves	18	245.1	1.4061N.S	18	65	.7756 N.S.
Varieties X Shelves	15			15		

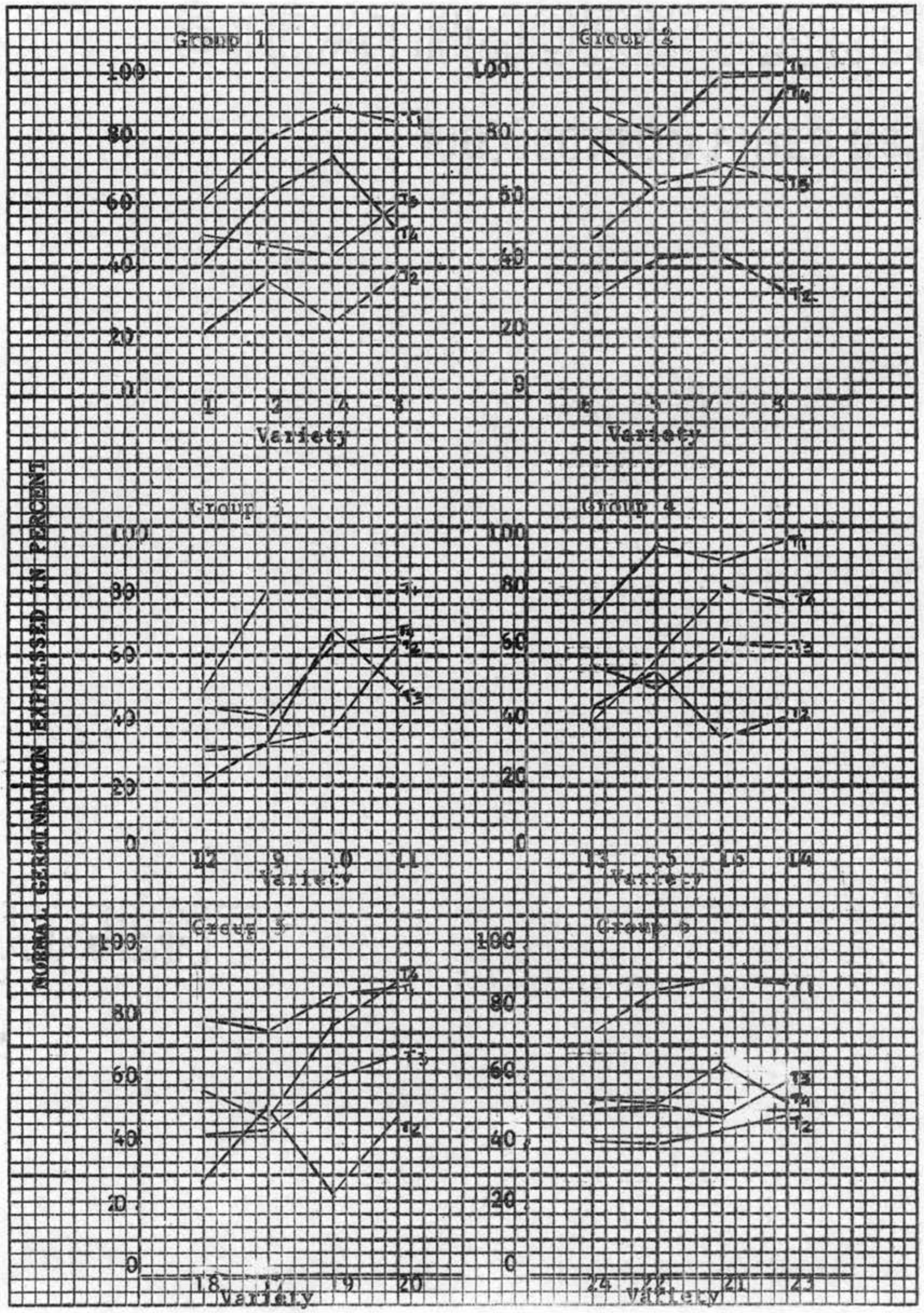

Figure 1. Relationship of normal germination response to treatment by variety and group.

The comparison of the average number of normal seedlings obtained on kimpak to the average obtained when the seedlings were required to exert some energy to emerge through a 1-inch layer of sand or vermiculite is striking. This indicates that emergence energy in sorghum is definitely harmed by the use of such treatments and is directly related to a weakened physiological condition of the seeds. This is evidently caused by the inbibition of moisture at cool temperatures.

The weight of the layer over the seed-zone apparently does not effect germination and emergence. The results obtained, which showed that emergence on an average was greater when the seeds were over-lain with sand than with vermiculite, support this hypothesis; however differences in water absorption capacity existed between these two substrates. In sand, water moved freely downward and was concentrated in the lower portion of the layer; while in vermiculite, water was absorbed readily and was more uniformly distributed throughout the seed zone. This may have influenced the results obtained with vermiculite.

A five-second exposure of the seeds to hot-water (100°C.) as in treatment 4, followed by germination on kimpak tissue compared favorably with the results of the 1-inch layer of sand over the seeds and pre-chilled for 5-days prior to germination. The seeds handled in this manner were partially cleansed of fungi which were especially evident when seeds were pre-chilled on kimpak. The response to the hot-water treatment gave some indication as to a possible separation between the varieties in respect to categories of vigor. The average germination of two varieties (8 and 20) was 90 percent and above. In comparison, four varieties had an average germination of 75 to 88 percent, seven germinated 60 to 74 percent, while the average germination of the remaining 11 varieties ranged from 41 to 69 percent as shown in Figure 1. An index for vigor apparently can not be determined by variety, for any treatment other than the hot-water test.

The lack of a significant interaction between treatment and varieties within groups 1, 2, 5 and 6, as shown in Figure 2, indicated these varieties have a similar pattern of response to treatments. The varieties within groups 3 and 4 were significantly different in response to treatment. In these two groups as measured by germination response, the four treatments behaved differently from variety to variety.

Relationship of Treatment to Speed of Germination of Varieties by Groups

The speed of germination is an accumulative index expressed in percent for each variety, derived by taking the number of normal seedlings per 25 seed units counted each day, times the reciprocal in days required.

Then the speed of germination of varieties by groups is plotted by treatments as shown in Figure 3, it would appear that the speed of seed germination in treatment 1 was by far the greatest regardless of variety in all groups. This is somewhat misleading however if interpreted superficially, since the seeds in this treatment had the advantage of a 5-day pre-chill treatment at 10^{o}C. which permitted imbibition of water, making them ready for immediate germination when placed in the warm environment.

The speed of germination, when plotted by treatment and variety for groups 4, 5 and 6, appeared similar to the curves for normal germination but on a lower scale. In general, the speed of germination when used as an index of vigor, decreased the values obtained for the varieties and treatments in all groups 20 to 40 percent as compared to the measure of vigor determined by normal germination.

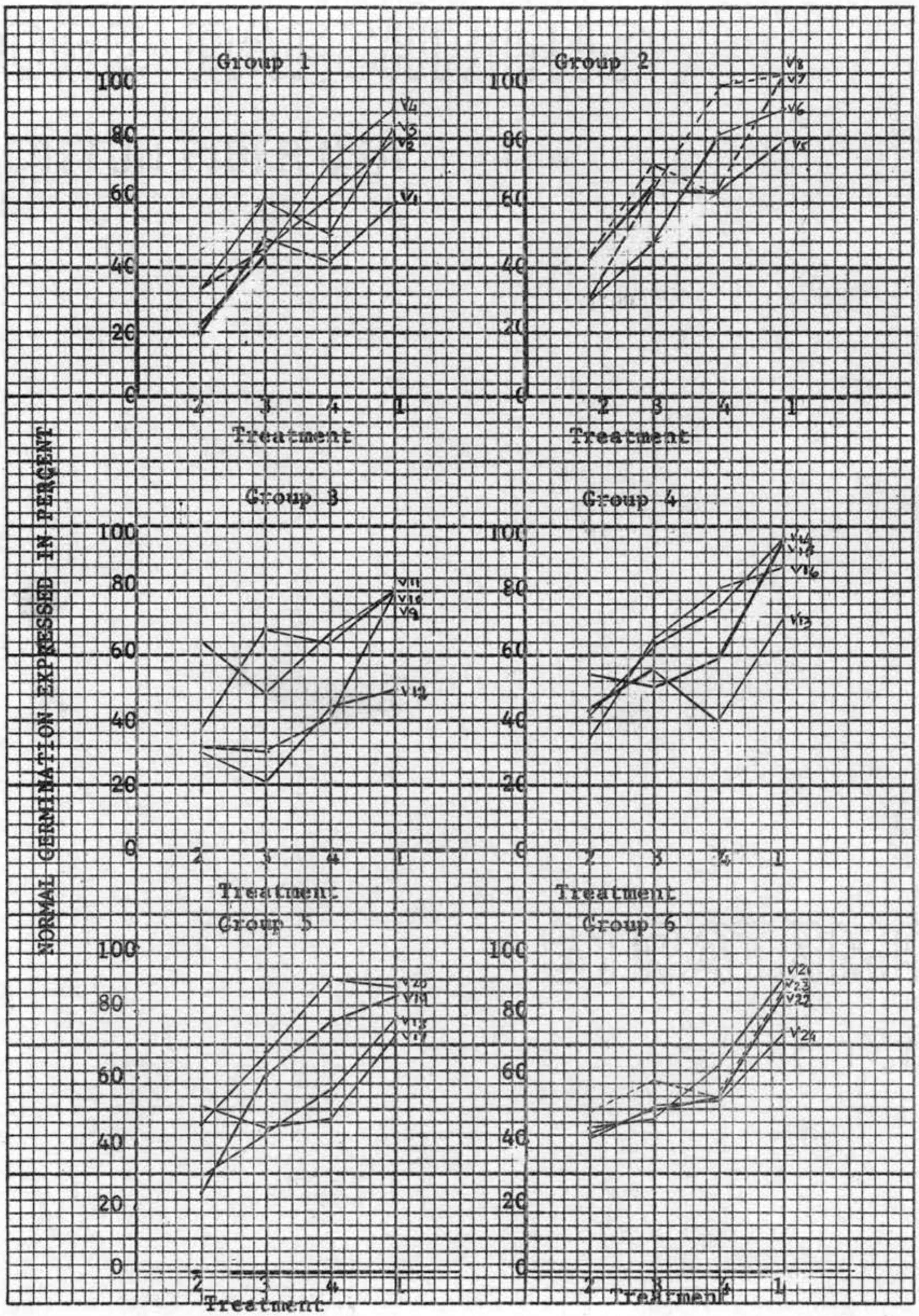

Figure 2. Relationship of normal germination response by variety to treatment in group.

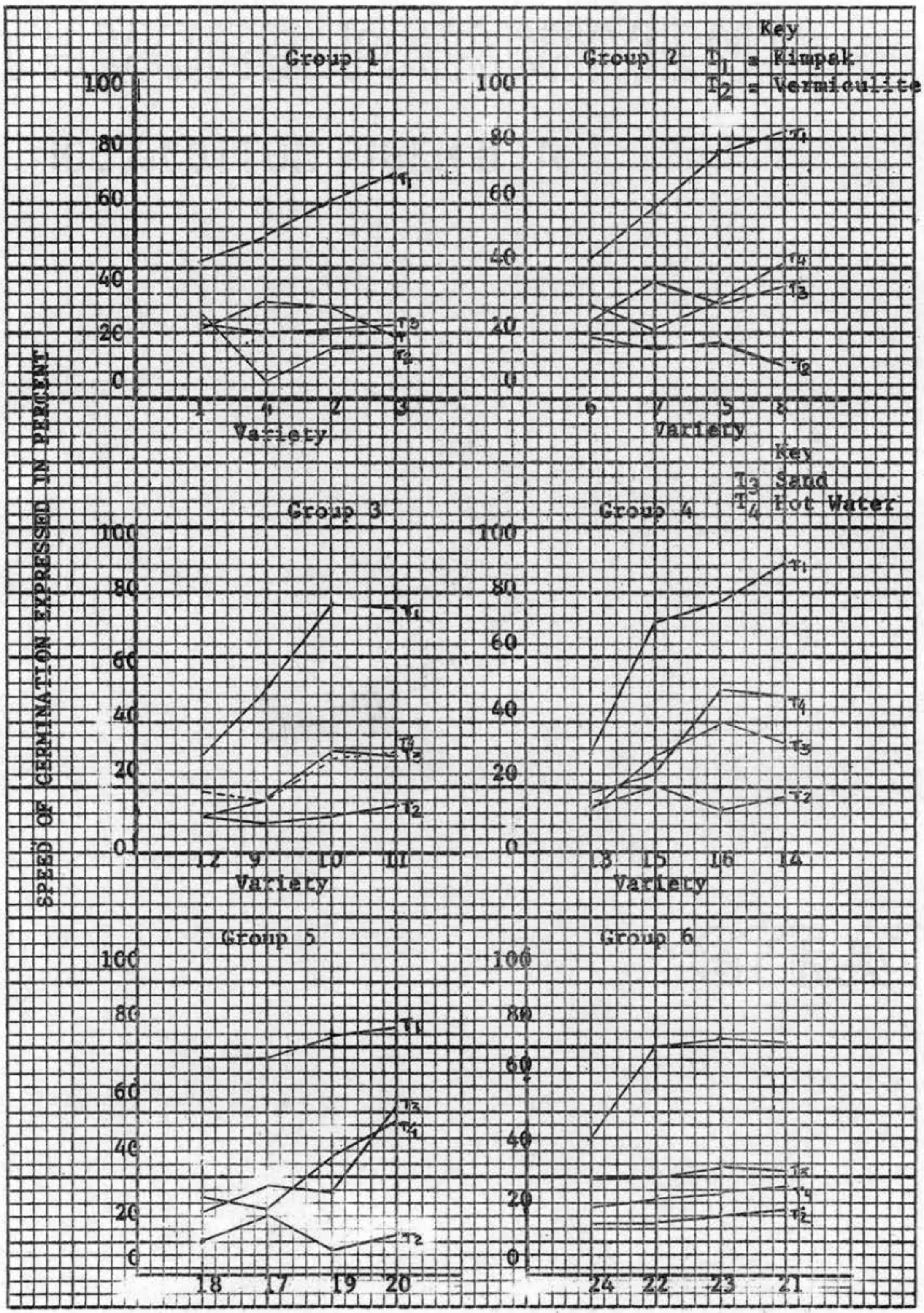

Figure 3. Relationship of treatment to speed of germination of varieties by group.

From the measures of vigor, as determined by speed of germination, it would appear that treatment 4 is perhaps the best indicator of seed vigor between varieties in a group. The basis for this statement was the close similarity to treatments 2 and 3 which measures the ability of seedlings to emerge from a depth of one inch. The differences between varieties in response to treatment is shown in Figure 4.

The varieties consistently low in vigor as measured by the speed of germination index, regardless of groups, were 6, 12, 13 and 24. The trend in response to treatment was very similar for all varieties.

Vigor as Measured Objectively by Variety in Groups as Influenced by Treatment

Vigor, as determined by objective measurements of the growing seedling at the end of a 5-day period of study (Figure 5) was not materially different in the pattern of response, for varieties by treatments, from that obtained by the other measurements of vigor (Figures 1 and 3). Differences in vigor did exist between varieties and also between treatments within varieties.

The largest differences in vigor between varieties were found to exist in the response patterns of treatment 4 (Figure 6). With the exception of varieties 6 and 12, the highest number of vigorous seedlings observed were in treatment 1. Most of the varieties (1, 2, 3, 4, 5, 6, 9, 11, 19, 20, 21, 22, 23, and 24) appeared to react to treatments in a like manner.

No attempt was made to correlate the speed of germination, normal percent germination and the objective measure of vigor. The statistical design was such that a correlation could not be made with any reasonable degree of confidence.

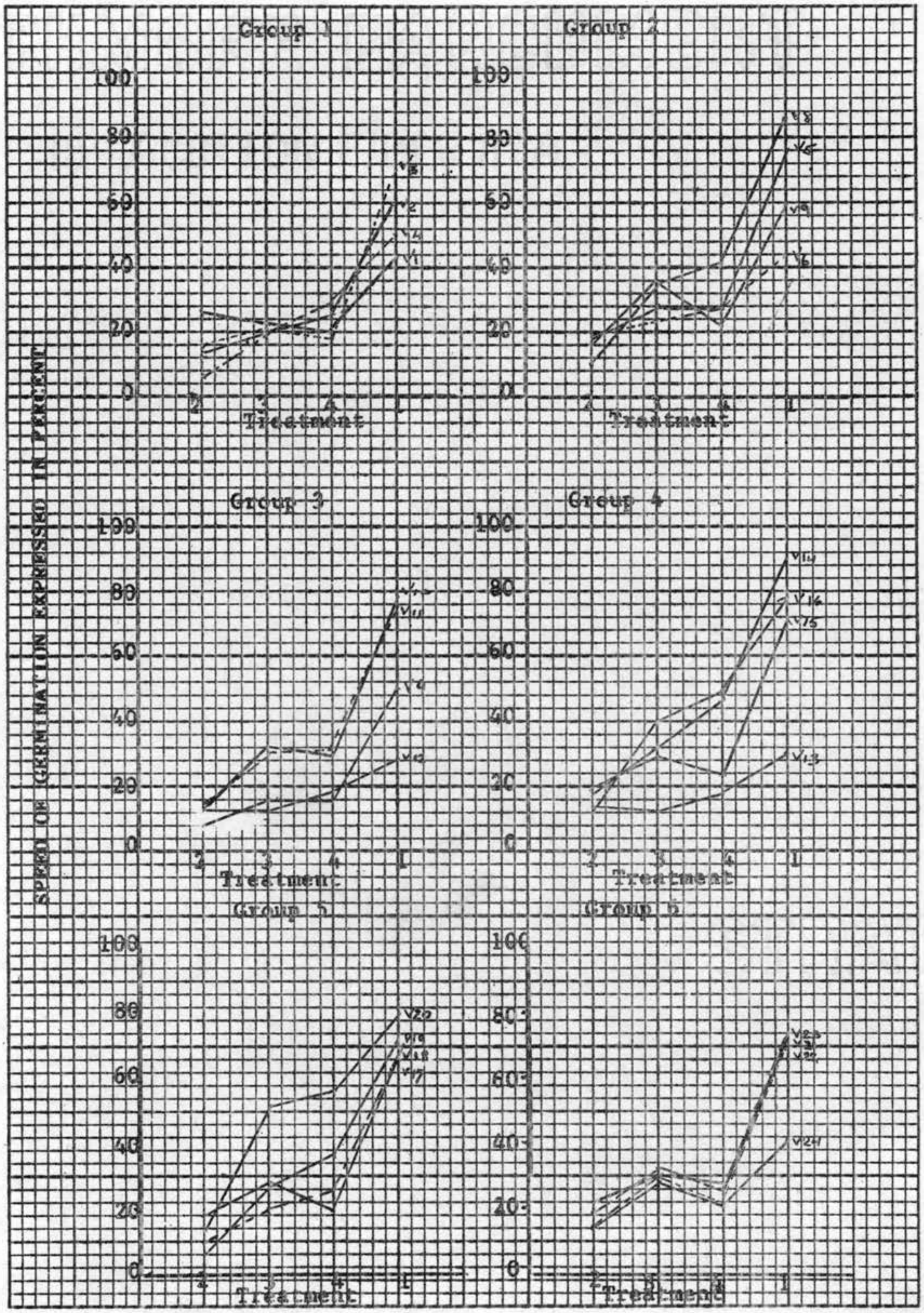

Figure 4. Relationship of speed of germination of variety to treatment by group.

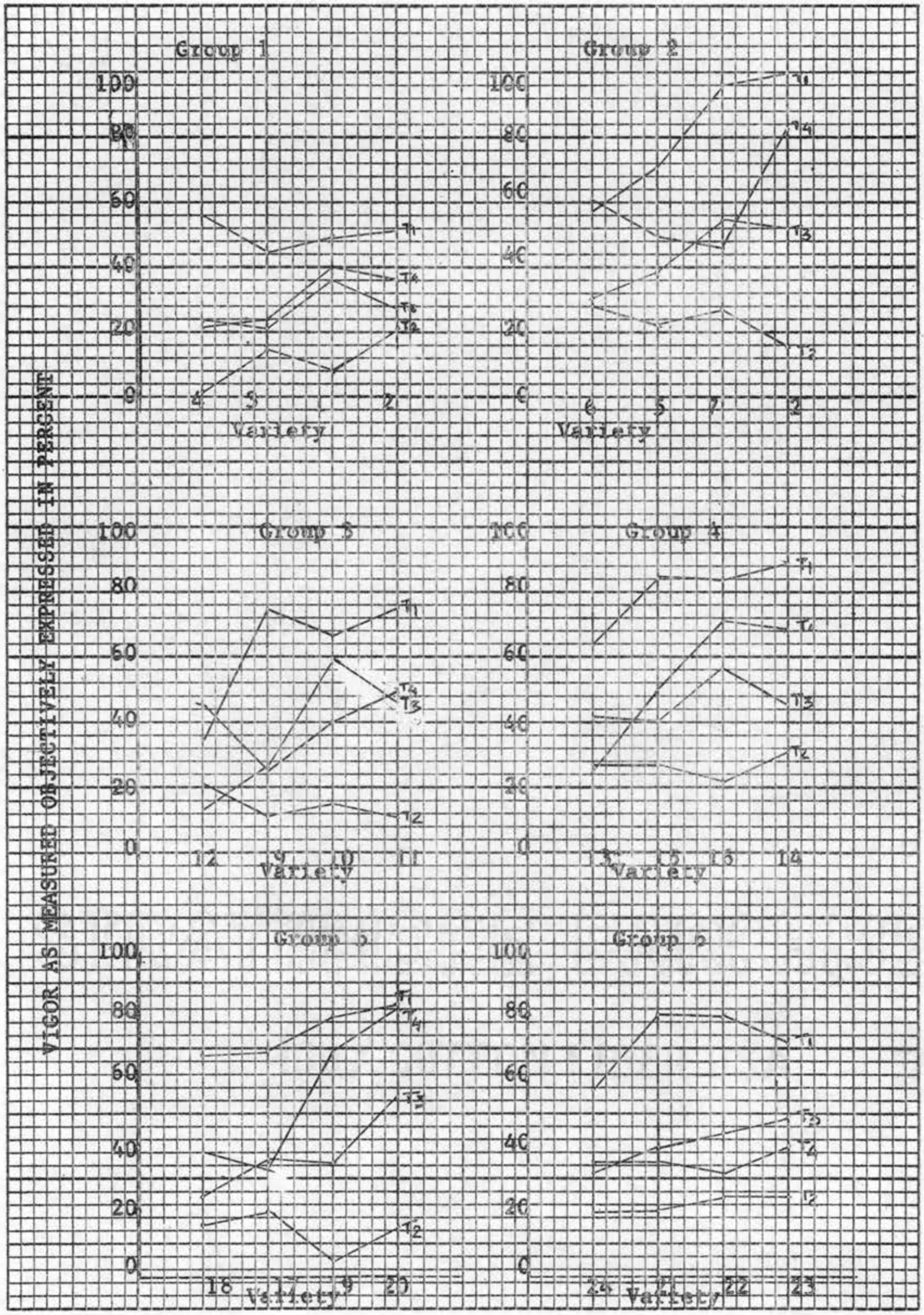

Figure 5. Vigor as measured objectively by variety in groups as influenced by treatment.

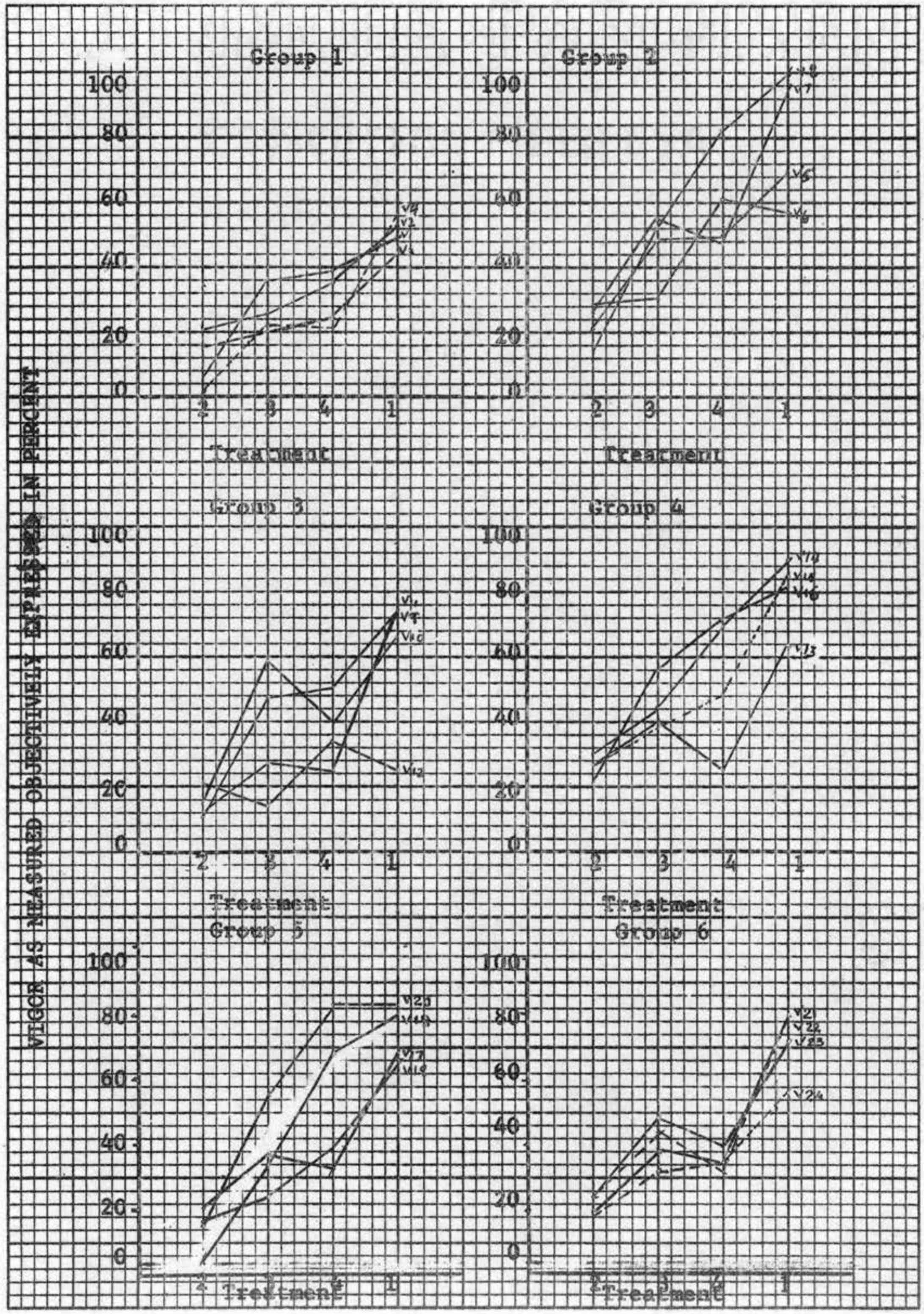

Figure 6. Relationship of variety to treatment in groups as objectively measured by vigor.

SUMMARY AND CONCLUSIONS

The objective of this study was to evaluate different laboratory techniques for determining seed vigor in forage and grain sorghum varieties. These investigations were conducted in 1962 using the facilities of the U.S.D.A. Grass Seed Research Laboratory, Oklahoma State University, Stillwater, Oklahoma.

Twenty-four accessions of sorghum varieties and hybrids were included in this study. These accessions consisted of both forage and grain types that are presently produced, or are being evaluated experimentally in Oklahoma.

Since there is not a standard vigor test recommended for sorghum, four treatments were included in this study. The principal phase of the cold test for seed corn, which is to subject the seeds to low temperatures (10°C.) for five days prior to transfer to a warm germination environment, was used in three treatments. All treatments used six thicknesses of kimpak in the bottom of the germination boxes. Treatment 2 contained a 1-inch layer of vermiculite and treatment 3, a 1-inch layer of sterile sand. The fourth treatment consisted of a 5-second exposure of the seeds to hot (100°C.) water.

Because of the large number of germination boxes required and the problem of incubation space, it was necessary to germinate one replicate at a time at 5-day intervals. The statistical design employed was an unusual incomplete block.

Significant differences were found among treatments replications, and shelves. Within the six-groups, significant differences among varieties were found in three groups, and in two of these significant interactions were measured among varieties and treatments. Differences among varieties and treatments within a variety were measured by both the speed of germination and the objective vigor measurements.

The measurements of vigor were found to be a very relative factor with results being closely associated with the type of substrate used.

In every case the order of rank by treatment per group, in percent normal germination was 1, 4, 3, 2. One-inch moistened layers of sand or vermiculite over the seed zone plus a 5-day prechill treatment at 10°C. before transfer to optimum conditions, reduced normal germination by as much as 75.3 percent. The data indicate that emergence energy in sorghum is definitely harmed by the use of such treatments, and is evidently related to a weakened physiological condition of the seeds. Apparently this was caused by the imbibition of moisture at a cool temperature. A similar situation existing under field conditions would have a drastic effect on subsequent stands.

The results obtained indicate that emergence was not affected by the weight of the layer over the seed zone. This theory is supported by the comparative differences found between seedling emergence when the seeds were over-lain with sand and vermiculite.

A five-second exposure of the seeds to hot water (100°C.) followed by germination on kimpak tissue, was the only treatment that gave an indication that the varieties studied differed in respect to vigor. The fungal cleansing effect of the hot water seed treatment may have, in part, attributed to the differences found.

When the speed of germination was used as an index of vigor by variety the response to treatment was similar to that of normal germination, but on a reduced scale. Since the speed of germination is an accumulative value for each variety, derived by taking the number of seeds to initiate germination each day times the reciprocal in days required; perhaps it should be expected that such a rating system would result in rather low values. From the measurements of vigor, as determined by speed of germination, treatment 4 appeared to be the best measure. This conclusion was based upon the close similarity of treatment 4 to treatments 2 and 3 which measures the ability of seedlings to emerge from a depth of one inch.

Vigor, as determined by objective measurements of the growing seedlings at the end of a 5-day period, was not materially different in the pattern of response for varieties by treatments from that obtained by the other measurements. The largest differences in vigor between varieties were found to exist in the response patterns of treatment 4. Most varieties appeared to respond to the other treatments in a like manner.

The numerical values obtained, as indicative of seed-vigor, were highest for all varieties when subjected to treatment 1. Although treatment 1 resulted in the highest numerical response when a one inch layer of sand or vermiculite was placed over the seed zone a reduction in vigor of as much as 75.3 percent was recorded.

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A P P E N D I X

TABLE IV

AVERAGE NORMAL PERCENT GERMINATION BY TREATMENT AND VARIETY

	Varieties	Treatments: Kimpak	Vermiculite	Sand	Hot water
Group 1	1	61.3	20.C	49.3	42.0
	2	80.0	36.0	46.6	62.6
	3	85.3	37.3	60.0	50.6
	4	89.3	22.6	45.3	73.3
Group 2	5	81.3	42.6	65.3	64.0
	6	89.3	30.6	48.0	81.3
	7	98.6	44.0	72.0	64.6
	8	100.0	30.6	66.6	94.6
Group 3	9	80.0	34.0	33.3	41.3
	10	80.0	37.3	68.0	64.0
	11	80.0	64.0	49.3	66.6
	12	49.3	32.0	21.3	44.0
Group 4	13	73.3	44.0	57.3	40.0
	14	96.0	41.3	62.6	77.3
	15	94.6	54.6	50.7	58.7
	16	89.3	34.6	65.3	81.3
Group 5	17	74.7	50.7	44.0	46.7
	18	77.7	29.3	42.7	56.0
	19	85.3	24.0	60.0	77.3
	20	89.3	47.2	66.6	90.0
Group 6	21	90.7	44.0	46.6	65.3
	22	86.6	41.3	50.6	52.0
	23	89.3	49.3	58.7	53.3
	24	74.0	41.6	50.0	53.3
Average		83.5	40.1	53.3	62.5

TABLE V

AVERAGE SPEED OF GERMINATION BY TREATMENT AND VARIETY

	Varieties	Kimpak	Vermiculite	Sand	Hot water
Group 1	1	43.40	26.72	22.08	21.52
	2	61.32	14.38	20.88	27.20
	3	69.00	15.98	22.52	18.64
	4	49.00	7.20	20.08	29.44
Group 2	5	75.76	17.20	28.32	28.96
	6	42.80	17.76	23.64	29.52
	7	59.00	15.08	36.32	22.32
	8	86.64	10.64	35.44	40.64
Group 3	9	50.76	8.40	15.76	15.76
	10	76.48	12.76	32.08	28.64
	11	75.00	14.64	30.44	30.64
	12	28.44	12.96	13.44	18.44
Group 4	13	29.76	13.64	13.44	18.44
	14	88.60	17.28	33.00	46.76
	15	72.20	19.76	28.08	24.32
	16	77.08	12.76	39.64	48.88
Group 5	17	65.64	17.88	27.44	20.44
	18	65.64	10.20	18.64	24.64
	19	72.96	7.20	25.08	35.96
	20	78.64	13.32	51.44	57.44
Group 6	21	71.32	21.08	31.44	26.84
	22	70.04	14.88	30.44	22.52
	23	73.32	18.08	33.44	24.64
	24	41.64	14.84	29.00	21.08

TABLE VI

AVERAGE PERCENT VIGOROUS SEEDLINGS AS MEASURED AT THE END OF A 5-DAY GERMINATION PERIOD BY TREATMENT AND VARIETY

	Varieties	Treatments: Kimpak	Vermiculite	Sand	Hot water
Group 1	1	50.64	6.64	36	38.64
	2	53.32	21.32	26.64	36.00
	3	45.32	14.64	21.32	24.00
	4	56.00	1.32	22.64	22.4
Group 2	5	70.64	21.32	49.32	49.32
	6	57.32	28.00	30.64	61.32
	7	96.00	26.64	54.64	46.64
	8	98.64	14.64	52.00	82.64
Group 3	9	74.64	12.00	26.64	25.32
	10	66.64	14.64	53.64	40.00
	11	74.64	10.64	46.64	49.32
	12	25.32	21.32	13.32	24.64
Group 4	13	64.00	26.64	41.32	25.32
	14	89.32	30.64	45.32	69.32
	15	85.32	26.64	40.00	48.00
	16	82.64	21.32	56.00	72.00
Group 5	17	69.32	21.32	37.32	33.32
	18	66.64	16.00	25.32	40.00
	19	80.00	5.32	34.65	68.00
	20	82.64	14.64	57.32	82.64
Group 6	21	81.32	20.00	38.64	34.64
	22	80.00	24.00	44.00	32.00
	23	72.00	24.00	49.32	40.00
	24	57.32	18.64	32.00	34.64
Total		1679.64	441.88	923.61	1090.12
Av.		69.96	18.40	38.48	45.50

TABLE VII

NORMAL PERCENT OF GERMINATION OF SORGHUM VARIETIES BY TREATMENT PER SHELF AND REPLICATION

	Replication - 1						Replication - 2						Replication - 3					
	Shelf - 3			Shelf - 5			Shelf - 2			Shelf - 12			Shelf - 3			Shelf - 8		
	Treatment						Treatment						Treatment					
Var.	2	3	Total	1	4	Total	1	2	Total	3	4	Total	2	4	Total	1	3	Total
1	32	60	92	80	72	152	56	4	60	44	48	92	24	48	72	48	44	92
2	68	64	132	96	92	188	76	32	108	60	56	116	8	40	48	68	16	84
3	60	69	128	84	96	180	92	36	128	80	44	124	16	12	28	80	32	112
4	56	44	100	100	52	152	80	4	84	44	96	140	8	72	88	88	48	136
Total	216	236	452	360	312	672	304	76	380	228	244	472	56	172	228	284	140	424
Av.	54	59		90	78		76	19		57	61		14	43		71	35	
	Shelf - 2			Shelf - 8			Shelf - 4			Shelf - 10			Shelf - 1			Shelf - 7		
	2	3	Total	1	4	Total	1	2	Total	3	4	Total	1	3	Total	2	4	Total
5	68	64	132	84	12	156	88	20	108	76	68	144	72	56	128	40	52	92
6	40	44	84	100	84	184	80	20	100	60	80	140	84	40	124	32	80	112
7	72	88	160	100	60	160	96	44	140	64	86	150	100	64	164	16	48	64
8	68	80	148	100	96	196	100	4	104	48	96	144	100	52	152	20	93	112
Total	248	276	524	384	312	696	364	88	452	248	330	578	356	212	568	108	272	380
Av.	62	69		96	78		91	22		62	82.5		89	53		27	68	
	Shelf - 10			Shelf - 12			Shelf - 6			Shelf - 9			Shelf - 6			Shelf - 14		
	1	4	Total	2	3	Total	3	4	Total	1	2	Total	2	4	Total	1	3	Total
9	84	64	148	28	40	68	32	32	64	80	8	88	36	28	64	76	28	104
10	96	60	156	72	64	136	76	76	152	76	28	104	12	56	68	68	64	132
11	72	72	144	44	60	104	40	60	100	92	44	136	28	68	96	76	48	124
12	48	60	108	20	24	44	28	52	80	72	44	116	32	20	52	28	12	40
Total	300	256	556	164	188	352	176	220	396	320	124	444	108	172	280	248	152	400
Av.	75	64		41	47		44	55		80	31		27	43		62	38	

TABLE VII (Continued)

	Shelf - 1			Shelf - 5			Shelf - 8			Shelf - 13			Shelf - 5			Shelf - 10		
	1	4	Total	2	3	Total	1	2	Total	3	4	Total	1	3	Total	2	4	Total
13	80	64	148	36	56	92	72	28	100	52	32	84	68	64	132	68	20	88
14	100	72	172	56	88	144	96	28	124	44	88	132	92	56	148	40	72	112
15	96	62	168	62	52	124	100	64	164	60	48	108	88	40	128	28	56	84
16	96	84	180	48	84	132	84	28	112	52	76	128	88	60	148	28	84	112
Total	372	296	668	212	280	492	352	148	500	208	244	452	336	220	556	164	232	391
Av.	93	74		53	70		88	37		52	61		84	55		41	58	
	Shelf - 4			Shelf - 13			Shelf - 5			Shelf - 11			Shelf - 9			Shelf - 13		
	2	3	Total	1	4	Total	3	4	Total	1	2	Total	2	4	Total	1	3	Total
17	60	52	112	76	68	144	44	36	80	60	52	112	40	36	76	88	36	124
18	44	76	120	88	64	152	24	28	52	64	16	80	28	76	104	80	28	108
19	48	76	124	84	88	172	56	84	144	96	8	104	16	60	76	76	48	124
20	68	96	164	96	100	196	48	92	140	84	24	108	36	78	114	88	56	144
Total	220	300	520	344	320	664	172	240	412	304	100	404	120	250	370	332	168	500
Av.	55	75		86	80		43	80		76	25		30	62.5		83	42	
	Shelf - 6			Shelf - 11			Shelf - 1			Shelf - 3			Shelf - 2			Shelf - 11		
	2	3	Total	1	4	Total	1	2	Total	3	4	Total	2	4	Total	1	3	Total
21	48	60	108	96	84	180	100	52	152	36	64	100	32	48	80	76	44	120
22	52	56	108	100	80	180	88	44	132	40	28	68	28	48	76	72	56	128
23	56	68	124	92	80	172	92	48	140	48	44	92	44	36	80	84	60	144
24	52	80	132	84	76	160	84	37	121	36	48	84	36	36	72	44	44	88
Total	208	264	472	372	320	692	364	181	545	160	184	344	140	168	308	276	204	480
Av.	52	66		93	80		91	45.25		40	46		35	42		69	51	

TABLE VIII

SPEED OF GERMINATION OF SORGHUM SEED BY VARIETY PER TREATMENT BY SHELF AND REPLICATION.

	Replication 1						Replication 2						Replication 3					
	Treatments						Treatments						Treatments					
	Shelf - 3			Shelf - 5			Shelf - 2			Shelf - 12			Shelf - 3			Shelf - 8		
Var.	2	3	Total	1	4	Total	1	2	Total	3	4	Total	2	4	Total	2	3	Total
1	2.3	5.83	8.13	14.05	5.5	19.55	7.5	.5	8.0	5.66	5	10.66	2.75	5.66	8.41	11	5.08	16.08
2	5.9	6.91	12.81	15	9.25	24.25	16	3.83	19.83	7	6.33	13.33	1	4.83	5.83	15	1.75	16.75
3	5.8	6	11.08	13.75	8.41	22.16	23.5	3.91	27.41	7.33	4.75	12.08	2.25	.83	3.08	14.5	3.58	18.08
4	4.41	4.16	8.57	14.75	4.25	19.00	11	.25	11.25	4.91	10.16	15.07	.75	7.66	8.41	11	6	17.0
Total	17.69	22.90	40.95	57.55	27.41	84.96	58	8.49	66.49	24.90	26.24	51.14	6.75	18.88	25.73	51.50	16.41	67.91
Av.	4.42	5.72		14.38	6.85		14.5	2.22		6.22	6.56		1.68	4.74		12.87	4.10	
	Shelf - 2			Shelf - 8			Shelf - 4			Shelf - 10			Shelf - 1			Shelf - 7		
	2	3	Total	1	4	Total	1	2	Total	3	4	Total	1	3	Total	2	4	Total
5	7.5	6.58	14.08	18.83	7.75	26.58	21.5	1.16	22.66	7.83	7.66	15.49	16.5	6.83	23.33	4.25	6.33	10.38
6	3.91	5.41	9.32	12.41	7.33	19.74	9.66	6	15.66	7.5	7.66	15.16	10	4.83	14.83	3.41	7.16	10.57
7	4.75	10	14.75	11.75	4.33	16.08	14.5	5	19.5	8.16	8.08	16.24	18	8.08	26.08	1.58	4.33	5.91
8	5.66	10.33	15.99	21.5	8.33	29.83	20	.25	20.25	9.75	11	20.75		6.50	30.0	2.08	11.16	13.24
Total	21.82	32.32	54.14	64.49	27.74	92.23	65.66	12.41	78.07	33.24	34.40	67.64	68	25.24	94.24	11.32	28.98	40.30
Av.	5.45	8.08		16.41	3.10		8.31	8.60		8.31	8.60		17	6.56		2.83	7.24	
	Shelf - 16			Shelf - 12			Shelf - 6			Shelf - 9			Shelf - 6			Shelf - 14		
	1	4	Total	2	3	Total	3	4	Total	1	3	Total	2	4	Total	1	3	Total
9	14.08	5.58	19.66	2.16	3.83	5.99	4.75	3.25	8.0	10.5	.58	11.08	3.58	3	6.53	13.5	3.25	16.75
10	22.38	5.75	28.13	5.83	7.5	13.33	9	9	18	19	2.75	21.75	1	6.75	7.75	16	7.58	23.58
11	17.25	5.83	23.08	4.16	11.0	15.16	6	6.33	12.33	20	4.58	24.58	2.25	10.83	13.08	19	5.83	24.83
12	8.50	5.58	14.08	1.83	5	6.83	3.75	5.75	9.50	9.33	4.91	14.24	3	2.5	5.5	3.5	1.33	4.83
Total	62.21	22.74	84.95	13.98	27.33	41.31	23.50	24.33	47.83	58.83	12.82	71.65	9.83	23.08	32.91	52	17.99	69.99
Av.	15.55	5.68		3.49	6.83		5.87	6.08		14.70	3.20		2.45	5.77		13.00	4.49	

TABLE VIII (Continued)

	Shelf - 1			Shelf - 9			Shelf - 8			Shelf - 13			Shelf - 5			Shelf - 10		
	1	4	Total	2	3	Total	1	2	Total	3	4	Total	1	3	Total	2	4	Total
13	14.83	5.66	20.49	3.08	10.08	13.16	17	2.91	19.91	7.75	3.5	11.25	11.16	10	21.16	7.41	3	10.41
14	20.91	13.0	33.91	6	7.25	13.25	23.05	3.08	26.13	10.50	12.91	23.41	22.5	7	29.5	3.88	9.16	13.04
15	15.83	6.08	21.91	6.33	8.66	14.99	22.33	6	28.33	7.33	5.83	13.16	16	5.08	21.08	2.5	6.33	8.83
16	23.33	10.83	34.16	3.66	15.58	19.24	19	3.08	22.08	6.16	13.5	19.66	15.50	8	23.50	2.83	12.33	15.16
Total	74.90	35.57	110.47	19.07	41.57	60.64	81.38	15.07	96.45	31.74	35.74	67.48	65.16	30.08	95.24	16.62	30.82	47,44
Av.	18.72	8.89		4.76	10.39		20.34	3.76		7.93	8.93		16.29	7.52		4.15	7.70	

	Shelf - 4			Shelf - 13			Shelf - 5			Shelf - 11			Shelf - 9			Shelf - 13		
	2	3	Total	1	4	Total	3	4	Total	1	2	Total	1	3	Total	1	3	Total
17	4.58	6	10.58	16.5	7.08	23.58	8.5	4.5	13	14.5	4.83	19.33	4	3.75	7.75	18.25	6.08	24.33
18	3.25	7.66	10.91	17.75	5.58	23.33	2.83	3.16	5.99	15	1.5	16.5	2.91	9.75	12.66	16.50	3.5	20
19	3.66	7.25	10.91	19.33	8.75	28.08	6.41	10.58	16.99	17.91	.75	18.66	1	7.66	8.66	17.50	5.16	22.66
20	5.16	24	29.16	20.16	15	35.16	7	12.10	19.10	18.83	2.08	20.91	2.75	16	18.75	20	7.58	27.58
Total	16.65	44.91	61.56	73.74	36.41	110.15	24.74	30.34	55.08	66.24	9.16	75.40	10.66	37.16	47.82	72.25	22.32	94.57
Av.	4.16	11.22		18.43	9.10		6.18	7.58		16.56	2.29		2.66	9.29		18.06	5.58	

	Shelf - 6			Shelf - 11			Shelf - 1			Shelf - 3			Shelf - 2			Shelf - 11		
	2	3	Total	1	4	Total	1	2	Total	3	4	Total	2	4	Total	1	3	Total
21	8.25	14.50	22.75	22.5	6.83	29.33	16	5.08	21.08	4.08	8.16	12.24	2.5	5.16	7.66	15	5	20
22	4.25	10.50	14.75	18.75	7.33	26.08	18.3	4.33	22.63	4.83	2.41	7.24	2.6	7.16	9.76	15.5	7.5	23
23	4.41	10	14.41	17.41	8.50	25.91	18.58	5	23.58	5.83	5.50	11.33	4.16	4.5	8.66	19	9.25	28.25
24	4.58	11	15.58	8.58	6.25	14.83	11.91	3.66	15.57	4.25	5.25	9.50	2.91	4.33	7.24	10	6.5	16.5
Total	21.49	46	67.49	67.24	28.91	96.15	64.79	18.07	82.86	18.99	21.32	40.31	12.19	21.15	33.32	59.50	28.25	87.75
Av.	5.37	11.5		16.81	7.22		16.19	4.51		4.51	5.33		3.04	5.28		14.87	7.06	

TABLE IX

OBJECTIVE MEASUREMENT OF SORGHUM SEED VIGOR BY VARIETY PER TREATMENT BY SHELF AND REPLICATION.

	Replication 1						Replication 2						Replication 3					
	Shelf - 3			Shelf - 5			Shelf - 2			Shelf - 12			Shelf - 3			Shelf - 8		
Var.	2	3	Total	1	4	Total	1	2	Total	3	4	Total	2	4	Total	1	3	Total
1	1	8	9	12	10	22	12	1	13	9	11	20	3	8	11	14	10	24
2	9	9	18	12	11	23	14	6	20	8	11	19	1	5	6	14	3	17
3	6	5	11	7	10	17	12	2	14	7	8	15	3	-	3	15	4	19
4	--	-	--	5	5	10	18	-	18	7	2	9	1	10	11	19	10	29
Total	16	22	38	36	36	72	56	9	65	31	32	63	8	23	31	62	27	89
Av.	4	5.5		9	9		14	2.25		7.75	8		2	5.75		15.5	6.75	

	Shelf - 2			Shelf - 8			Shelf - 4			Shelf - 10			Shelf - 1			Shelf - 7		
	2	3	Total	1	4	Total	1	2	Total	3	4	Total	1	3	Total	2	4	Total
5	9	15	24	15	14	29	20	-	20	11	14	25	18	11	29	7	9	16
6	6	8	14	17	15	32	12	10	22	10	15	25	14	5	19	5	16	21
7	9	17	26	24	11	35	24	9	32	11	16	27	24	13	37	2	8	10
8	8	16	24	24	22	46	25	-	25	10	19	29	25	13	38	3	21	25
Total	32	56	88	80	62	142	81	19	100	42	64	106	81	42	123	17	54	71
Av.	8	14		20	15.5	20.25	4.75		10.5	16		20.25	10.5		4.25	13.5		

	Shelf - 10			Shelf - 12			Shelf - 6			Shelf - 9			Shelf - 6			Shelf - 14		
	1	4	Total	2	3	Total	3	4	Total	1	2	Total	2	4	Total	1	3	Total
9	20	13	33	3	8	11	6	3	9	18	1	19	5	3	8	18	6	24
10	22	12	34	7	15	22	15	14	29	12	3	15	1	4	5	16	17	30
11	22	10	32	3	13	16	10	10	20	15	3	18	2	17	19	19	12	31
12	5	12	17	3	4	7	4	9	13	10	6	16	7	5	12	4	2	6
Total	69	47	116	16	40	56	35	36	71	55	13	68	15	29	44	57	34	91
Av.	17.24	11.75		4	10		8.75	9		13.75	3.25		3.75	7.25		14.25	8.5	

TABLE IX (Continued)

	Shelf - 1			Shelf - 9			Shelf - 8			Shelf - 13			Shelf - 5			Shelf - 10		
	1	4	Total	2	3	Total	1	2	Total	3	4	Total	1	3	Total	2	4	Total
13	15	9	24	3	10	13	15	5	21	11	5	16	17	10	27	12	5	17
14	21	19	40	10	9	19	24	6	30	11	17	29	22	14	36	7	16	23
15	22	11	33	8	10	18	24	10	34	14	12	26	18	6	24	2	13	15
16	24	17	41	6	19	25	18	5	23	9	18	27	20	14	34	5	19	24
Total	82	56	138	27	48	75	82	26	108	45	52	97	77	44	121	26	53	79
Av.	20.5	14		6.75	12		20.5	6.5		11.25	13		19.25	11		6.5	13.25	

	Shelf - 4			Shelf - 13			Shelf - 5			Shelf - 11			Shelf - 9			Shelf - 13		
	2	3	Total	1	4	Total	3	4	Total	1	2	Total	2	4	Total	1	3	Total
17	6	10	16	18	12	30	11	7	18	14	6	20	4	6	10	20	7	27
18	4	10	14	20	11	31	5	6	11	16	2	18	4	13	17	14	4	8
19	3	11	14	20	20	40	6	17	23	21	1	22	-	14	14	19	9	28
20	4	23	27	23	24	47	8	20	28	18	2	20	3	18	21	21	12	33
Total	17	54	71	81	67	148	30	50	80	69	11	80	11	51	62	74	32	106
Av.	4.25	13.5		20.25	16.75		7.5	12.5		17.25	2.75		2.75	12.75		18.5	8	

	Shelf - 6			Shelf - 11			Shelf - 1			Shelf - 3			Shelf - 2			Shelf - 11		
	2	3	Total	1	4	Total	1	2	Total	3	4	Total	2	4	Total	1	3	Total
21	5	14	19	22	10	32	20	8	28	6	10	16	2	6	8	19	9	28
22	6	14	20	23	13	36	20	8	28	6	5	11	4	6	10	17	13	30
23	3	12	15	18	14	32	17	8	25	11	8	19	7	8	15	19	14	33
24	6	8	14	15	12	27	17	4	21	5	7	12	4	7	11	11	11	22
Total	20	48	68	78	49	127	74	28	102	28	30	58	17	27	44	66	47	113
Av.	5	12		19.5	12.25		18.5	7		7	7.5		4.25	6.75		16.5	11.75	

VITA

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